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NUTRITION REVIEWS

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Published Monthly at Mt. Royal and Guilford Aves., Baltimore, Md.

for

THE NUTRITION FOUNDATION, INC.

99 PARK AVE. NEW YORK 16, N. Y.

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Rate $4.00 per year in the United States and Canada; $4.50 in other countries.
Changes of address should be sent to The Nutrition Foundation.
Second-class postage paid at Baltimore, Md.

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NUTRITION REVIEWS

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NUTRITION, ANTHROPOLOGY, AND THE STUDY OF MAN

If anthropologists have much to learn from the specialized studies of nutrition, nutritionists also have something to gain from the anthropologist. If anthropologists gain from a consideration of the cultural significance of food habits, perhaps nutritionists could profit from wider speculations on man and civilization.

In growing proof of this, recent years have seen increasing interaction between the fields of anthropology and nutrition. Anthropology is concerned with the development of man and his behavior; nutrition is involved in understanding the specific relationship of man and his behavior on the one hand, and the elements of the environment that nourish him on the other. It is worthwhile, periodically, to review areas of common interest to better understand the nature of the benefits being derived from this association, and to re-examine the potentialities.

The history of man has been one of continuing adjustments to different environments, and diet is one of the central factors in the adjustments of the organism to an environment. If a now extinct giant ape (Pei Wen-Chung, *Am. Anthrop.* **59,** *834 (1957)*) was carnivorous, and contemporary apes are essentially herbivorous and insectivorous, why is it not possible for different societies of men, over the millenia of human development, to make markedly different adjustments to different environments? Certainly many racial characteristics, such as the sub-cutaneous fat of the mongoloid and the lankiness of the Nilotic Negro can be attributed to long termed environmental adaptations (C. S. Coon, S. M. Garn, J. B. Birdsell, *Races*, Springfield (1950); R. Linton, "Crops, Soils and Culture in America", *The Maya and Their Neighbors*, N. Y., 1940, pp. 32–40; C. Collazos, *Arch. venezol. nutricion* **5,** *343 (1954)*; *Nutrition* **28,** *273 (1944)*).

The study of man is replete with one-sided points of view. The development of man and civilization has variously been attributed to the topography, latitude, climate, skin color, individual stature, technology, systems of production, kinds or numbers of gods, and even the bean. Few students of man are sufficiently specialized in more than a few disciplines to give adequate attention to more than a few sets of factors. Even though the past fifty years of anthropology generally favor attributing man's development to multiple and complex factors, every once in a while someone proposes a particularly suggestive correlation between some single factor and human or cultural development.

In terms of diet as a factor, why cannot the entire organism respond to different diets in appropriate ways, so that we do not have merely different diets for men, but rather different diets for different men? Nutritionists in the so-called backward areas are increasingly aware of this; nutritionists in the centers of scientific study, however, seem to be less aware of it. If this general view is correct, it means that the nutritionist's task is, in a sense, multiplied by the number of distinct environment-diet-races of man that can be identified. Each of these races may have quite a different cultural adjustment to a given environment.

For example, nutritionists in Guatemala have been concerned over the infant malnutrition that produces such spectacular conditions as kwashiorkor; at the same time nutritionists in general claim concern with the optimum condition of man. The anthropologist, looking at the Guatemalan Indian, is impressed with the infant malnutrition, but he is equally impressed with the ability of the adult Indian male, small and wiry, to carry a load of 100 pounds or more (so heavy, in fact, that he cannot hoist it alone)

for three or four consecutive days. The anthropologist, whose most extensive physical effort may be walking half a mile to his office, can only marvel at the ability of the Indian to maintain a condition that seems surprisingly optimum in terms of the demands of his life. Similarly, the experiences of Stefansson with the aboriginal pure meat diet of the Eskimos, while flying in the face of some current findings of scientific nutrition, does demand an explanation. The anthropologist is seldom in a position to give an explanation for either the Guatemalan corn and bean based capacity, or for the Eskimo's meat based survival. Eventually, the nutritionist must contribute to this.

Thus, it seems that anthropologists and nutritionists can benefit from mutual consideration of the behavior of man through the ages.' Oddly enough, however, the most characteristic contact between anthropologist and nutritionist has not been on this high level of scientific concern, but in the much more prosaic issue of how to get cooperation between one kind of man, the applied nutritionist or dietician, and another, the member of a society.

Applied nutritionists, unlike their scientific brethren, are not concerned with the fine details of biochemical intake and reactions, but with the simple issues of whether children get enough milk or appropriate protein. On this level, as well, the relationship between the fields of anthropology and nutrition can be a two-way affair.

Through the gradual identification of applied nutrition as a public health activity, the applied nutritionist has begun to turn to the anthropologist (and other social scientists as well) to find better ways to institute changes of food habits in population segments manifesting dietary deficiency diseases. The anthropologist's functional and wholistic approach is of particular utility in trying to find ways to change dietary habits. He is trained to see the human habit as part of a complex pattern of behaviors, involving dynamic motivations and positive reasons for existence. Where the child is not given milk throughout a population, it is not regarded by the anthropologist as ignorance, but as a manifestation of some positive satisfaction that the members of the population achieve from acting as they do. Therefore, the issue is not one of filling a void that is ignorance, but of changing one positive habit to another.

When the anthropologist becomes involved in a task such as this, he finds himself working not merely with food habits of a subject population, but with the complex social relations that exist between the applied nutritionist, other public health personnel, and members of the society in which they are operating. The anthropologist finds that changing the work patterns and thinking habits of the professional personnel is a necessary prerequisite for reaching the food habits of the subject population. In the experience of the writer, these are equally difficult.

The vested interest of the applied public health worker in his activities and methods is quite as strong as is the interest of the population in retaining their favored food habits. Anthropological work of this kind, then, frequently becomes a matter of dealing with the organization of public health work, for it is here that the habits of public health workers are manifest.

Once communication is established between the applied nutritionist and anthropologist in these situations, the latter also becomes the learner. The nutritionist who distinguishes between socially distinct segments of a population in which he is making a dietary survey brings out information of inestimable value to the anthropologist. The nutritionist's information on the kinds of changes or differences in diet occurring between population segments varying in wealth, in general cultural traditions, or in other social characteristics, provides the anthropologist with a precise kind of data that he lacks either the time or capacity to collect himself. The nutritionist who clearly

identifies certain psychological characteristics with nutritional conditions, such as the dullness of kwashiorkor children, provides the anthropologist with insights into the culture of the people he is trying to understand.

The most fruitful interaction between the fields of nutrition and anthropology can be derived through a recognition that each has perspectives and areas of understanding that are not common to both. While something is to be gained through the training of specialists of one field in the other, most scientific advancements will come from specialists who are competent in their own discipline, but who can broaden their vision in order to recognize the significance of the findings and perspectives of the other.

Both at the scientific level (understanding what food man really needs) and the applied level (understanding how to help man get the food he does need) there is a broad and productive future for the collaborative efforts of anthropology and nutrition.

RICHARD N. ADAMS, PH.D.
Professor of Sociology & Anthropology
Michigan State University
East Lansing, Michigan

THE PROGNOSIS IN JUVENILE OBESITY

Obesity is not only an important nutritional problem in adults in this country, but also has become an important nutritional problem among school children. More than ten per cent of the school children in two communities investigated were obese (*Nutrition Reviews* **15,** *6 (1957)*). Although long-term follow-up studies on the prognosis of obesity in childhood have not yet been carried out, a retrospective study of the role of juvenile obesity in adult obesity has recently appeared.

A. G. Mullins (*Arch. Dis. Child.* **33,** *307* (1958)) recorded the age, sex, height and weight in 373 consecutive patients in a British medical out-patient department. One hundred one of these patients were 20 per cent or more overweight when compared with the standard tables of W. F. F. Kemsley (*Ann. Eugen. Lond.* **16,** *316* (1952)).

Among these patients, those that had been known "fatty" at school (the most practical criterion) were classified as a juvenile obese group. The obese group included 26 per cent of the men and 44 per cent of the women. Approximately one-third of the obese group were cases of persistent juvenile obesity and included five men and 27 women. When the degree of obesity was classed as mild, moderate, or severe depending on the relative per cent overweight, the juvenile obese group made up 53 per cent of those classified as severely obese, but only 12 per cent of those with mild obesity.

For comparative purposes a "normal" group of 50 patients within 20 per cent of normal weight were studied in a similar way. These were chosen primarily for cooperation and intelligence and cannot be considered a control group. Where 50 per cent of the juvenile obese group had obese fathers and 22 per cent were married to obese people, the incidence in the normal group was 2 per cent and 4 per cent, respectively. Obese mothers were relatively common, being reported in 38 per cent of the normal group and 60 per cent of the juvenile obese group. The group developing obesity in adult life was intermediate between the other two but tended to resemble more closely the juvenile obese group. It was also observed that the juvenile obese group gained less weight during the physiological changes of pregnancy and the menopause than other types of obesity.

The results of treatment of these patients, by getting them accustomed to a bulky low-calorie diet and re-educating their eating

habits on an out-patient basis, were also analyzed. Among the juvenile obese group, one-quarter had lost more than 30 per cent of their excess weight during a four month period but there were no good results, defined as reduction to within 20 per cent of standard weight. Good results were achieved in 14 per cent of the adult obese group during the same period of time. It was also observed that the adult obese group with manifest psychological problems were more easily treated than those not having obvious problems. Among the juvenile obese group those with manifest psychological problems proved to be most refractory to therapy.

This retrospective study suggests that persistent juvenile obesity makes up a very significant proportion of adult obesity, and that the juvenile obesity patients tend to predominate among those adults who are severely obese. The fact that adults who have been obese since childhood are more difficult to treat becomes a tremendous challenge for those responsible for the medical care of school age children.

It would not be unreasonable to suppose that the prevention of obesity in this age group would be followed by significant reduction in the incidence of obesity in the adult population, with a consequent reduction in the mortality associated with extreme overweight. Obesity among school age children must be recognized as an important public health problem.

IONIZED CALCIUM AND PARATHYROID HORMONE

Calcium is present in the plasma in three forms, ionized, protein bound, and complexed, probably as a citrate. It is believed that the three fractions are in equilibrium with each other and, on determining serum calcium by the usual techniques, the total calcium content is determined. The determination of ionized calcium, until recently, has been dependent on a bioassay involving a frog heart (F. C. McLean and A. B. Hastings, *J. Biol. Chem.* **107,** *337* (1934)).

Recently, A. G. Rose (*Clin. Chim. Acta* **2,** *227 (1957)*) proposed a chemical method of determining the level of ionized calcium in serum. It is based on the ultrafiltration of serum under a known carbon dioxide tension. This technique is much simpler than the bioassay.

It has been proposed that many of the physiologic effects of calcium are closely related to the level of ionized calcium (*e.g.*, tetany, caused by low calcium levels, may disappear when the patient becomes acidotic even though he may have no change in the serum total calcium level and may reappear under conditions tending to increase the blood pH). Also, it has been suggested that the level of ionized calcium is very dependent upon the activity of the parathyroids. However, many of these conclusions are based on little direct evidence, mainly because the bioassay for ionized calcium has found somewhat limited use.

The calcium in the serum of 17 patients with proven parathyroid adenoma was fractionated, recently, by H. M. Lloyd and G. A. Rose (*Lancet* **II,** *1258 (1958)*). In 13 patients, there were determinations done both pre- and postoperatively. In addition, studies were carried out on a healthy volunteer who was given parathyroid hormone.

The 17 patients ranged from 23 to 60 years of age and consisted of four males and 13 females. It was of interest to note that nine of the patients showed renal stones on roentgenographic examination and, in addition, four more showed nephrocalcinosis. All, except one of the patients, had at least one serum calcium value which approached 11 mg. per 100 ml. and the plasma urea concentration usually varied between 24 and 40 mg. of urea per 100 ml.

The normal limits for ionized calcium, using the technique of Rose, were found to

be 5.9 to 6.5 mg. per 100 ml. of plasma water. The amount of calcium found bound to protein fell within the range of 2.4 to 3.9 mg. per 100 mg. of plasma water. In the 17 cases of known parathyroid hyperactivity, the ionized calcium ranged from 6.1 to 9.5 mg. per 100 ml. of plasma water, with only two patients having ionized calcium levels below 7.1 mg. per 100 ml. of plasma water (one was 6.8 and the other was 6.1). Thus, in patients with increased parathyroid activity, the plasma ionized calcium is increased.

On the other hand, if 3.9 mg. of protein bound calcium per 100 ml. of plasma water were considered as the upper limits of normal, then, of the 17 patients studied with hyperparathyroidism, seven had values below this level, preoperatively, and three additional patients had protein bound calcium levels between 4.0 and 4.2 mg. per 100 ml. of plasma water. Thus, in ten of the 16 patients, the level of protein bound calcium was within normal limits or only slightly above.

In addition to preoperative measurements, in 12 of the patients, these investigators obtained levels of total plasma calcium and both the ionized and protein bound fractions, postoperatively. These studies were carried out from three to fourteen days postoperatively with most tests conducted from six to eight days after the operation. In all instances, there was a drop in total plasma calcium averaging about 3 mg. per 100 ml. of plasma water, with a range of 0.1 to 7.2 mg. The ionized calcium, also, fell postoperatively with an average decrease of 2.8 mg. per 100 ml. of plasma water. In contrast, there was little or no change in the protein bound calcium, postoperatively. In fact, increases were noted in this fraction in four patients following the operation. On the average, the protein bound calcium fell about 0.5 mg. per 100 ml. of plasma water. Thus, in this study, it was noted that patients who had increased parathyroid activity due to an adenoma, had increased levels of ionized calcium in the plasma and that a prompt decrease in ionized calcium levels was found, postoperatively. The decrease in the ionizable calcium following the operation is considerably greater than any decrease noted in the protein bound calcium.

F. C. McClean and A. G. Hastings (*J. Biol. Chem.* **108,** *285 (1935)*) proposed the view that the ratio of concentration of calcium ions times protein ions divided by the concentration of calcium proteinate in plasma was constant. From this formula, any increase in calcium ion concentration should be accompanied by an increase in the protein bound fraction. The results of the study of Lloyd and Rose indicate that there were large changes in the ionized calcium in the plasma of patients with increased parathyroid activity with or without corresponding changes in the levels of protein bound calcium. This is at odds with the view of McLean and Hastings. It has, also, been reported earlier by J. Hellstrom (*Cleveland Clin. Quart.* **20,** *253 (1953)*) that three patients with hyperparathyroidism, had normal serum calcium levels and increased serum ionized calcium levels. The presence of increased levels of ionized calcium with a normal level of the protein bound calcium is explainable by either of the two following conditions; a) a decrease in plasma protein levels or b) a decreased ability of the plasma proteins to bind calcium, which might be a function of pH. In the cases reported by Lloyd and Rose, there were normal levels of plasma protein and the pH of the blood was usually in the normal range (although the blood pH was determined by calculation from the alveolar CO_2). These investigators suggested that the parathyroid hormone modifies the plasma proteins so that they are able to hold less calcium. How the hormone might change the plasma protein's affinity for calcium is not known.

To obtain further information concerning the effect of parathyroid hormone on the ability of plasma proteins to bind calcium, a healthy volunteer was given injections of parathyroid hormone. The total calcium in

his plasma rose from 10.2 mg. per 100 ml. of plasma water to 12.1 following these injections. At the same time, the ionized fraction increased from 6.0 to 7.5 mg. per 100 ml. of plasma water with the protein bound calcium increasing proportionally, 3.9 to 4.5 mg. per 100 ml. of plasma water. There was also an increase in the plasma protein level, from 6.2 to 7.7 g. per 100 ml., because the subject developed polyuria and became dehydrated. The increase in plasma protein concentration made the increase in the protein bound calcium less than expected. This study suggested to these investigators that the parathyroid hormone caused an increase in the plasma ionized calcium, but a decrease in the calcium binding capacity of the proteins.

The detection of hyperparathyroidism in man presents many difficulties, and unless there is a marked elevation of the serum calcium level (usually greater than 11.5 mg. per 100 ml.) and other frank signs, the differentiation of this disorder is difficult. In many cases, the occurrence of a renal stone may be the first manifestation of the disorder, with the levels of calcium and phosphorus in the blood at normal or near normal values. Lloyd and Rose suggest that the determination of ionized calcium in plasma may have diagnostic value, particularly in patients who have normal total plasma calcium levels.

Summary: The determination of the plasma ionized calcium levels was carried out in patients with a proven parathyroid adenoma and found to be elevated. The level fell following the removal of the adenoma. The decrease in ionized calcium postoperatively, was far greater than that noted for the protein bound calcium. It was believed that the parathyroid hormone influences the ability of the plasma protein to bind calcium.

LIPEMIA CLEARING AFTER VEGETABLE AND ANIMAL FATS

Two groups of workers have demonstrated the presence of active heparin in human plasma (L. Freeman, H. Engelberg and A. Dudley *Am. J. Clin. Path.* **24,** *5* (*1954*); I. M. Nilsson and A. Wenckert *Acta Med. Scandinav.* **150,** *supp., 297* (*1954*)). P. F. Hahn (*Science* **98,** *19* (*1943*)) observed that heparin injected into experimental animals caused clearing of post-alimentary lipemia. E. D. Korn (*J. Biol. Chem.* **215,** *15* (*1955*)) proposed that heparin served as a co-factor or component of an enzyme called lipoprotein lipase. The nature of the clearing factor and the mechanism of action of heparin have been reviewed recently (*Nutrition Reviews* **12,** *294* (*1954*)).

The role of nutritional and hormonal factors on the level of plasma fatty acids has also been summarized (*Nutrition Reviews* **14,** *216* (*1956*)). Injection of protamine and toluidine blue causes elevation of serum lipoproteins (J. E. Hewitt, *et al.*, *Cardiologia* **21,** *353* (*1952*); J. H. Bragdon and R. J. Havel *Am. J. Physiol.* **177,** *129* (*1954*)) and delay in chylomicron removal (J. E. French, B. Morris and D. S. Robinson *Proc. of Third International Congress on Biochemical Problems of Lipids, 323* (*1956*)) implying the neutralization of heparin and lipemia clearing factor.

H. Engelberg (*Proc. Soc. Exp. Biol. Med.* **97,** *304* (*1958*)) found an increase in circulating heparin in 12 of 18 individuals following the intravenous administration of a vegetable fat emulsion.

Because of these facts and since animal and vegetable fats differ in their effect on serum cholesterol and beta lipoprotein, H. Engelberg (*J. Applied Physiol.* **13,** *296* (*1958*)) investigated the effect of oral ingestion of animal and vegetable fats on the heparin level and lipemia clearing activity in man. The subjects were 21 healthy, young adults of both sexes. Blood samples

were taken in the fasting state and two to three hours after drinking 240 ml. of cream or an equal quantity of a safflower oil emulsion. Ten individuals from a previous study did not have determinations after vegetable oil.

The assay was done by precipitation of the protein bound heparin with a methanol-acetone mixture, digestion with trypsin to release the protein and decrease the molecular size, removal of the enzyme by heat coagulation and removal of the protein fragments by dialysis. Finally the dialysate was lyophilized and taken up in saline for the determination of anticoagulant activity. The method used was a micro modification of the U.S.P. 14 method for heparin assay.

Lipemia clearing activity was determined in a spectrophotometer by observing the rate of change in optical density of a mixture of plasma with a small amount of coconut oil emulsion.

After the ingestion of butter fat, the levels of heparin were unchanged in ten, decreased in two, and increased in nine individuals. Following administration of vegetable fat, levels of heparin were increased in two, decreased in two, and unchanged in seven.

Lipemia clearing activity after cream was increased in four, decreased in four, and unchanged in 13 patients. After oral ingestion of safflower oil, clearing increased in two, decreased in four, and remained unchanged in five subjects. It is evident that these clearing factor changes did not parallel the changes in heparin values.

The delay in clearance of postalimentary lipemia seen in atherosclerotic patients, along with the fact that substitution of vegetable or unsaturated fats for animal or saturated fats decreases serum lipids and cholesterol, suggested to H. Engelberg that vegetable fat might be a more effective activator of the heparin-lipemia clearing system. The results of the study did not confirm this postulate. Neither plasma heparin values nor lipemia clearing were increased following oral intake of safflower oil. Although nine of 21 individuals showed increased heparin levels after animal fat, the changes were suggestive rather than statistically significant. The fact that intravenous fat caused a more definite rise in plasma heparin suggested that this was due to the rapid entrance of fat into the circulation and the attainment of higher levels of lipemia.

The effect of an intravenous fat emulsion on plasma lipemia clearing ability was the subject of an earlier study reported by H. Engelberg (*J. Applied Physiol.* **12,** *292 (1958)*). Ten hospitalized patients with no hematologic or lipid metabolic disorder were given intravenous fat emulsion (Lipomul) for one hour after drawing a control blood sample. Two samples were taken 15 and 60 minutes after the start of the infusion and a third sample was collected about 60 minutes after stopping the treatment. A similar schedule of blood sampling was followed in individuals receiving intravenous glucose and in six persons after oral fat intake.

Since clearing activity continues uninterrupted at room temperature, blood was drawn in chilled syringes and kept cool during preparation for the test. As previously described, the best estimate of clearing ability is derived from the greatest rate of clearing seen in any ten minute trial rather than over the interval of an hour. The production of inhibitory end products or calcium soaps that might obscure continued decreases in optical density necessitate this type of evaluation.

Clearing activity was present in the fasting blood samples of most of the ten subjects. During the intravenous feeding, clearing activity decreased in seven, increased in two, and remained unchanged in one patient. One hour after completing the infusion, lipemia was absent from the plasma samples, but at this same time the clearing factor was at control levels in four patients, below the fasting level in three, and above in three.

During the entire two hour period of measurement the clearing activity was increased in four patients, and reduced in eight either during or after the infusion.

In order to determine whether the increase in clearing factor could be elicited nonspecifically, a 5 per cent glucose solution in half normal saline was given intravenously to four of the ten subjects. Two of the four patients whose clearing factor had increased after intravenous fat showed no change after glucose was given. In the other two a slight increase or decrease may have occurred. Six patients given cream orally showed a decrease in clearing factor in four, and an increase in two. Two subjects investigated before and after drinking skim milk showed no change in clearing factor.

In seven of the ten subjects various inhibitors of clearing activity were tried and in all cases there was complete or partial inhibition by sodium chloride, tetraethylpyrophosphate, sodium taurocholate, or protamine sulfate. These inhibitors do not affect pancreatic lipase activity. Tricalcium phosphate gel, which selectively adsorbs clearing factor but not pancreatic lipase, failed to reduce plasma clearing activity in one of the seven patients investigated.

The results of this study using an intravenous fat emulsion suggest that lipemia clearing activity is inactivated in the process of clearing. G. H. Jeffries (*Quart. J. Exp. Physiol.* **39,** *261* (*1954*)) and J. A. Spitzer (*Am. J. Physiol.* **185,** *18* (*1956*)) found that post-heparin clearing factor is inactivated by the liver. Accordingly, several factors may be responsible for the amount of clearing activity that may be detected at any one time, namely the extent of its stimulation and release, its inactivation by the liver, and the lipolytic process.

Summary: The effect of oral ingestion of cream or safflower oil emulsion on plasmic heparin and lipemia clearing activity was studied. Although the trends were not clear-cut, oral cream appeared to stimulate the release of heparin into the blood stream. Neither type of fat was the more effective in stimulating lipemia clearing activity. The effect of the intravenous infusion of fat on clearing activity was two-fold. Lipemia clearing was frequently stimulated by the presence of fat but also seemed to be inactivated during the clearing process.

PRIMARY HYPEROXALURIA AND OXALOSIS

"Primary hyperoxaluria" has received little attention in the United States; a single review has appeared in this journal in the past nine years (*Nutrition Reviews* **16,** *75* (*1958*)). Although only one or two dozen cases are said to have been reported, increased awareness of the condition will probably lead to more frequent recognition.

H. E. Archer, A. E. Dormer, E. F. Scowen and R. W. E. Watts (*Brit. Med. J.* **I,** *175* (*1958*)) outline the natural history of this disease as "one of progressive bilateral calcium oxalate urolithiasis, nephrocalcinosis, and recurrent infection of the urinary tract . . . death occurs in childhood or early adult life from renal failure and hypertension. There are no characteristic overt biochemical abnormalities except for a continuous high urinary oxalate excretion which is unrelated to exogenous factors; and urinary oxalate determinations are necessary to distinguish these patients from cases of calcium oxalate urolithiasis without hyperoxaluria". Calcium oxalate is deposited in various tissues, especially the kidneys, where secondary infection is the rule.

In their earlier article (*Lancet* **II,** *320* (*1957*)), discussed in *Nutrition Reviews* (*loc. cit.*), the same authors had been able to find but ten acceptable cases in the literature; in

only three of these had the high oxalate excretion been demonstrated by chemical measurement. Renal calculi were first discovered in their two patients at the ages of one and four years, respectively.

J. T. Godwin, M. F. Fowler, E. F. Dempsey and P. H. Henneman have published an article entitled "Primary Hyperoxaluria and Oxalosis; report of a case and review of the literature" (*New Engl. J. Med.* **259,** *1099 (1958)*). Their patient had been well until the age of five, when he passed a renal calculus and was found to have stones in both kidneys. Thereafter he had "prolonged genito-urinary difficulties, with the passage of many stones and with recurring infections" leading to his death at the age of ten.

There was no history of renal calculi in the parents. A brother had had "acute glomerulonephritis" at six months of age; "he appeared to recover, though from time to time pus cells were noted in the urine". Bilateral renal calculi were demonstrated at age six. At six and one half years the brother "became ill of 'glomerulonephritis', anuria developed, and he died in a few days". There was no autopsy. An aunt had had a single large renal calculus removed at the age of 28. Eight years later (in 1958) she was well.

The patient was studied for ten days at the age of eight. He was small (height 121.5 cm., weight 20.6 kg.) but otherwise normal upon physical examination. No crystals could be found in the eyes. The urine contained albumin, white cells, red cells and calcium oxalate crystals. Several renal stones gave a four plus test for calcium oxalate. Six 24-hour urine specimens contained 130 mg. to 180 mg. of oxalate (normal 15 mg. to 50 mg. in 18 adults and two children). There were calcifications in the kidney pelves and calyxes; a renal function test was normal. Bone development was retarded. Four months later the boy was admitted to another hospital "for progressive renal failure". He survived this but was readmitted almost two years later, in terminal renal failure. Post-mortem examination showed kidneys of 40 g. and 60 g. with orange-yellow cortices and containing gritty material. The calyxes all contained orange-yellow stones.

Microscopic sections showed innumerable calcium oxalate crystals in glomeruli, in and about tubules and in interstitial tissue, as well as evidence of secondary infection. Calcium oxalate crystals were abundant in the thymus; there were a few in vertebral cartilage and rare crystals in various other tissues. None was seen in bone marrow. A portion of kidney yielded 31 g. of calcium oxalate per 100 g. of tissue. A calculus "consisted principally of calcium oxalate." Several good photomicrographs show the crystals under polarized light. A small focus of encephalomalacia was found; no explanation is given.

The authors' case is a welcome addition to the small list; unfortunately metabolic studies could not be carried out. There is a continuing series of such studies by Archer and his colleagues of the St. Bartholomew's Hospital group in London and, as more cases become available, one hopes that the matter will be elucidated.

There is, of course, a large body of literature about urinary oxalate in relation to stone formation, as oxalate stones are common in urologic practice. This literature has what might be called a urologic-biochemical orientation, rather than a metabolic one, and has scarcely touched on what the St. Bartholomew's Hospital group calls primary hyperoxaluria. However, articles are beginning to appear on this subject from various places.

Archer and co-workers point out that transient increases of oxalate excretion ("secondary hyperoxaluria") are well recognized after ingestion of oxalate-rich foods such as rhubarb, spinach, strawberries, cocoa and chocolate. The normal range of urinary oxalate excretion as determined by Archer's

group is only 10 to 40 mg. per 24 hours. But their two patients, for example, excreted 162 to 290 and 110 to 265 mg. per 24 hours respectively in many determinations over a two year period. These individuals were on a diet unrestricted but for the exclusion of oxalate-rich foods; their hyperoxaluria was not transient and probably dated from birth. When sodium oxalate was given to these two patients by mouth (*loc. cit.*, (*1958*)) the increase in urinary oxalate excretion was similar to that found in normal individuals (less than 5 per cent of the dose). Thus it is clear that the oxalate is not ingested, but is formed in the body. It seems quite proper to consider primary hyperoxaluria an entity. There is evidence to indicate that it may be familial, presumably an inborn error of metabolism.

The St. Barthlomew's Hospital group doubts that it is dealing with a primary renal defect (*i.e.* leakage of oxalate, leading to calculi, infection, kidney failure, oxalate retention and final tissue deposition), for oxalosis (tissue deposition) is not found in renal failure of other causes. They favor a metabolic theory; excessive formation of oxalate, causing high concentrations in body fluids with tissue deposits and excessive oxaluria leading to urinary stone formation with resulting recurrent infection and eventual renal failure and renal hypertension. A suitable method of measuring plasma oxalate concentration is sorely needed; such a technique would advance our knowledge considerably. One could then measure renal clearances in normal subjects and compare them with those in primary hyperoxaluria. Although the theory of a renal leak seems untenable, it is well not to deny the possibility of a biochemical lesion (of kidney tubules) in any disease where a tissue enzyme defect is suspected and plasma levels and tubular transport mechanisms have not yet even been studied. There are possible parallels between this situation and that in other inborn errors of amino acid metabolism which affect the kidney tubule (*Nutrition Reviews* **16,** *39* (*1958*)).

Archer and co-workers show a possible scheme implicating glycine:

$$\text{Glycine} \rightleftharpoons \text{glyoxylate} \rightarrow \text{formate} + \text{carbon dioxide}$$
$$\downarrow$$
$$\text{oxalate}$$

Thus, an excessive production of oxalate might result from an excess of glycine, or partial or total inability to transform glyoxylate to formate and carbon dioxide. The latter might be caused by an (inborn) enzyme lack. These investigators gave sodium benzoate in an effort to deplete the body glycine pool by conjugation and, indeed, found urinary oxalate excretion decreased; two normal subjects showed no such effect. When glycine was given to one patient with primary hyperoxaluria, oxalate excretion was apparently somewhat increased. A normal subject did not show this effect.

In order to prove the thesis that glycine is converted to oxalate, Scowen, J. C. Crawhall and Watts (*Lancet II, 300* (*1958*)) gave glycine-1-C^{13} by mouth to a patient with primary hyperoxaluria and report "prompt and considerable incorporation of the isotope into the urinary oxalate". No estimate was made of the proportion of oxalate derived from glycine nor of glycine converted to oxalate. With a substance as plentiful and capable of so many reactions as glycine, this will doubtless prove quite variable. Also, oxalate is very likely of more than one origin.

Calcium oxalate occurs in other conditions, not only in calculi, but in retinas, cataracts, thyroid and arteries. Godwin's group reviews some of this material and concludes with a bibliography of 34 references.

In summary, a case report of primary hyperoxaluria and oxalosis is reviewed. In this condition excessive oxalate excretion is associated with multiple renal calculi, starting in childhood, leading eventually to

death in renal failure in childhood or early adult life, with deposits of calcium oxalate in the tissues. Evidence is adduced that this entity may be an inborn error of metabolism.

CARBOHYDRATES AND FOOD INTAKE

It has been known for a long time that the type of dietary carbohydrate has a marked influence on the rat's requirement for various members of the vitamin B complex. (For review see O. Mickelsen, *Vitamins and Hormones* **14,** *1 (1956)*). It has been postulated that the more insoluble carbohydrates, such as the dextrins and starches, escape complete hydrolysis and serve as favorable media for the growth of bacteria in the lower part of the digestive tract. These bacteria synthesize the essential vitamins that are missing from the diet. These vitamins are then absorbed and permit the animal fed a deficient diet to grow.

More recently evidence has begun to accumulate that the type of carbohydrate in the diet has an effect on the amino acid requirement. M. W. Marshall and M. Womack (*Nutrition Reviews* **12,** *182 (1954)*) found that when adult rats were fed a purified ration containing each of the essential amino acids at a level of 0.1 per cent, the animals on the sucrose ration lost 28.8 mg. of nitrogen per day while those on the dextrin ration lost only 15.5 mg Adding glutamic acid or increasing the level of the essential amino acids to 0.2 per cent improved nitrogen balance but, in both cases, the animals on the dextrin ration did better than those on the sucrose.

Additional evidence for the superiority of complex carbohydrates over the simpler sugars in "sparing protein" comes from the work of the Wisconsin group. A. E. Harper and M. C. Katayama (*J. Nutrition* **49,** *261 (1953)*) showed that on a 9 per cent casein ration containing 0.5 per cent methionine, weanling rats gained 25 g. per week (average for the four-week period) when the carbohydrate was starch (other ingredients in per cent: corn oil 5, salts 4, and adequate vitamins). The growth rate was 10 to 13 g. per week when sucrose replaced the starch. On 18 per cent casein diets, the rats gained 35 and 38 g. on the sucrose and starch rations, respectively.

When the low casein-sucrose ration was supplemented with liver extract, extra vitamins, an antibiotic-vitamin B_{12} mixture or the unessential amino acids, there was no increase in growth rate. The addition of essential amino acids, which are limiting in a 9 per cent casein ration (0.4 per cent lysine, 0.15 per cent valine, 0.2 per cent threonine, 0.1 per cent tryptophan and 0.2 per cent histidine), increased the growth rate to that of the starch group (25 g. per week). A similar improvement in the growth of the rats fed the sucrose ration was seen when the level of casein was raised to 12 per cent. When dextrin (produced by heating moist corn starch in an autoclave at 15 pounds pressure for three hours) was used in place of sucrose, growth on the 9 per cent casein ration was the same as that with corn starch.

The hypotheses put forth to explain the superiority of complex carbohydrates over the simpler sugars were: 1. The less soluble carbohydrates favor the synthesis of the essential amino acids by the intestinal microflora; 2. improved digestion and absorption of protein occurred as the result of the slower passage of the complex carbohydrates through the gastrointestinal tract; 3. there was a reduced destruction of the amino acids in the animal as a result of the prolonged absorption of glucose from the insoluble carbohydrates (A. E. Harper and C. A. Elvehjem *J. Agricultural and Food Chemistry* **5,** *754 (1957)*).

To test the first of the preceding hypotheses, the Wisconsin investigators (H. E. Spivey *et al.*, *Am. J. Physiol.* **193,** *479 (1958)*) excised the cecum (the large blind pouch in which the large intestine begins) in 6 rats. This operation minimized the synthesis of essential nutrients by the intestinal microflora. The cecectomized rats grew as well as the sham-operated ones. But, for both of the preceding groups, the animals receiving the corn starch ration grew at a rate which was twice that of the sucrose-fed rats (23 versus 11 g. per week). The addition of 1 per cent sulfasuxidine to the corn starch diet-fed normal rats did not decrease the rate of growth. These findings strongly suggest that the intestinal flora could not explain the differential growth observed when rats are fed a low protein diet containing different carbohydrates.

The invalidity of the second hypothesis was shown by growth studies utilizing a maintenance level of a mixture of essential amino acids supplemented with ammonium citrate (Spivey *et al., loc. cit.*). If improved digestion and absorption of amino acids occurred as a result of the slower passage of complex carbohydrates through the intestinal tract, an improvement in nitrogen utilization should be seen when corn starch was the carbohydrate compared with sucrose. The rats receiving corn starch did gain slightly more weight during the four-week experiment than those receiving sucrose, but the difference was not statistically significant. There was, however, no difference in the per cent of nitrogen retained.

The third hypothesis was tested by training the animals to consume their daily food requirement in a period of one and one-half hours. One group of animals received the complete sucrose diet during the one and one-half hour feeding period, while the second received the same diet but with only 25 per cent of the sucrose. This group received the remaining sucrose as a solution by stomach tube, 25 per cent every two hours during the succeeding six hours. The control rats receiving the complete sucrose diet were stomach-tubed at comparable times in order to maintain the conditions as identical as possible.

Again, both groups of rats gained weight at approximately the same rate. This indicates that the slower absorption of glucose had no effect in enhancing the growth of rats on the low casein diet. For this reason, the investigators felt that the slower liberation of glucose from the complex carbohydrate, with a consequent sparing of amino acids, could not explain the differences in growth rates.

These observations led the investigators to conclude that it is "unlikely that any of the hypotheses previously proposed can account for the beneficial effect of dextrin in stimulating food intake and growth rate of rats consuming low protein diets."

In view of these results, the Wisconsin investigators sought an explanation for the better growth of dextrin-fed animals in some physical or chemical property of the carbohydrate. The osmotic pressure of the carbohydrates was the first property tested. For this purpose, A. E. Harper and H. E. Spivey (*Am. J. Physiol.* **193,** *483 (1958)*) studied the food intake of rats weighing 100 to 150 g. When rats were trained to eat all their food in one and one-half hours each day, there was no difference in the amount of food consumed during the first week by the rats receiving either dextrin or sucrose. Both groups lost about the same amount of weight (14 and 13 g. per week, respectively).

During the second week, the group receiving dextrin ate a little more and gained more than did the group receiving sucrose. From then on, the animals on the dextrin ration ate considerably more than the rats on the sucrose ration (8.8 versus 6.8 g. per day) and gained more rapidly (20 versus 11 g. per week). The rats receiving dextrin drank more water than those on sucrose (14.8 versus 11.6 ml. per day). This was unexpected, since the greater osmotic activity of sucrose should have led to an increased water consumption.

To determine whether comparable differ-

ences in water accumulation in the stomach occurred, rats that had been trained to eat 5 g. of food in 30 minutes were killed at intervals after eating. The per cent of moisture and the dry weight of the stomach contents were determined. Up to seven hours after the feeding period the sucrose-fed rats had considerably more material in their stomachs than the dextrin-fed rats. The stomach contents of the sucrose-fed rats was 1.2 times as great as that of the dextrin-fed rats one hour after feeding; the difference increased with time until the fifth hour, when the stomach contents of the sucrose-fed rats was two times that of the dextrin-fed rats. The difference in the size of the stomach contents was attributable almost entirely to water. The stomachs of the sucrose-fed rats had from 2 to 4 ml. more water in them than those of the dextrin-fed rats for the first five to seven hours after feeding. In spite of the differences in stomach contents, there was no essential difference in the rate at which solids left the stomachs of the two groups of rats.

This observation suggested to the investigators that "the type of carbohydrate consumed can influence stomach volume appreciably through its effect on the amount of water retained in the stomach. Stomach volume increased, whereas food intake and growth decreased, when a carbohydrate of low molecular weight was substituted in the diet for one of high molecular weight." Harper and Spivey (*loc. cit.*) postulated that the reason why rats grow equally well on a high protein diet, regardless of the type of carbohydrate is that "the replacement of some of the low molecular weight carbohydrate by protein which exerts less osmotic pressure" permits the rat to eat more and "that less of the more adequate diet is required to satisfy the amino acid requirements of the rat."

The reduction in osmotic pressure resulting from an increase in dietary protein may be partially responsible for the observed improvement in growth. It does not appear to be the only explanation, since calculation shows that increasing the casein from 9 to 19 per cent means a reduction in glucose from 80 to 70 per cent. This reduction of 12.5 per cent in dietary sucrose would not explain the increase of 22.7 per cent in food intake observed when the protein level was increased (6.8 versus 8.8 g. per day).

The Wisconsin investigators (A. Yoshida, A. E. Harper and C. A. Elvehjem (*J. Nutrition* **63,** *555 (1957)*) had observed that weanling rats fed low levels of casein grew more rapidly as the dietary fat was increased from 0 to 10 to 30 per cent. In each experiment, the protein to calorie ratio was the same for all of the diets. Nitrogen balance studies suggested that the greater weight gain and improved caloric utilization were attributable to increased food consumption and to the increased caloric density of the high fat diets.

To determine whether the preceding effects of the high fat diet were related to the carbohydrate in the diet, Yoshida, Harper and Elvehjem (*J. Nutrition* **66,** *217 (1958)*) fed weanling rats diets ranging in fat content from 1 to 30 per cent. All diets contained 25 mg. of protein per calorie. The dietary level of fat had no influence on the growth rates of the dextrin-fed rats during the first week; the rats on the 1, 10 and 30 per cent fat diets gained 36 g. The rats receiving either sucrose or glucose gained 21, 24 and 33 g. per week when fed rations containing 1, 10 and 30 per cent fat, respectively. Hydrogenated vegetable oil (oleomargarine) was the fat used. When the rats were trained to eat 5 g. of diet within 30 minutes, the stomachs contained 9.5 and 6.9 g. of material for the sucrose and dextrin-fed rats, respectively, one and one-half hours after feeding.

At this time (one and one-half hours) the fat content of the diet had no effect on the volume of the stomach. Three hours after feeding, the rats fed the sucrose ration with 1 per cent of fat had 8.4 g. in their stomachs, while the group fed a similar diet containing 30 per cent fat had 6.2 g. in their stomachs. The latter was approximately the same as

that of the group receiving dextrin. The increase in the stomach contents of the rats fed the low-fat sucrose ration was largely due to water.

The observation that the growth of rats receiving sucrose or glucose in the low protein diet was improved with additions of fat and there was no change in the growth rates of the dextrin-fed rats was attributed to the "capacity of the diet to exert osmotic pressure and thereby affect the amount of water retained in the stomach."

The above observations are interesting and intriguing since they introduce a new vector that must be considered in nutritional experiments. The work of the Wisconsin group emphasizes the fact that the potential osmotic pressure of the material in the stomach may have a profound effect upon food consumption and consequently upon growth rates.

It is recognized that the volume of the stomach plays some role in the regulation of food intake. However, under normal circumstances there is an adaptive mechanism which permits the animal to adjust his food intake to his requirement. This has been brought out by the studies of J. L. Strominger and J. R. Brobeck (*Yale J. Biol. and Med.* **25,** *383 (1953)*), who found that when an inert filler was added to the diet, rats rapidly adjusted the amount of food consumed so that the caloric intake remained constant.

Although no data are available from Strominger and Brobeck's work on the effect of the inert filler on stomach volume, their results suggest an increase in stomach capacity after the animals became adjusted to the high bulk diet. One would assume a similar adaptive mechanism should occur in the rats fed carbohydrates of different osmotic potential. If this did not occur, then it could be postulated that the animals might eat more frequently throughout the 24-hour period. It is hoped that further work to answer these questions will be forthcoming.

BIOTIN AND CELL PERMEABILITY

The exact physiological function of biotin is unknown. Since animals can grow at a normal rate without a dietary source of biotin, it is believed that this vitamin is synthesized by the bacteria in the intestinal tract (O. Mickelsen, *Vitamins and Hormones* **14,** *1 (1956)*). Additional support for this thesis is provided by the work at the University of Notre Dame with germ-free animals (*Nutrition Reviews* **14,** *116 (1956)*). The latter work showed that germ-free animals develop a typical deficiency of biotin when this vitamin was absent from the diet. A deficiency can be produced in conventional experimental animals only by the presence in the diet of avidin, a protein which combines with and prevents the absorption of biotin.

Although much of the earlier work with biotin was done with experimental animals, most of the recent studies have been with bacteria. These studies originated partly from the recognition that lipids interfered with a number of microbiological assays. The work of V. R. Williams and E. A. Fieger (*Indust. Eng. Chem. Anal. Ed.* **17,** *127 (1945)*) showed that the addition of 20 micrograms of oleic acid to the biotin assay medium increased the growth of *Lactobacillus casei* both in the presence and absence of biotin. The oil extracted from whole rice, and lecithin, had similar effects. The above work was extended by W. L. Williams, H. P. Broquist and E. E. Snell (*J. Biol. Chem.* **170,** *619 (1949)*) who reported that most of the lactic acid bacteria did not require oleic acid when grown on complete media. When biotin was omitted from the media, oleic acid was necessary for growth. They concluded that in the bacteria studied, the most

important function of biotin is in the synthesis of oleic acid because when oleic acid was present in the medium, biotin was apparently not required.

To get a clearer understanding of the importance of biotin to *Lactobacillus arabinosus*, A. Traub and H. C. Lichstein at the University of Minnesota (*Arch. Biochem. & Biophys.* **62,** *222 (1956)*) investigated the interrelationship between biotin and the unsaturated fatty acids. They found that in the presence of an optimal amount of biotin (0.01 micrograms per 6 ml.) the growth of the organism with small inocula was characterized by a lag period of 24 to 36 hours. The addition to the medium of a surfactant, or reduction of the initial pH from 6.8 to 6.0, reduced the lag period and produced a rapid and regular rate of growth.

That the lag period at pH 6.8 was due to a limited permeability of the cell to biotin was strengthened by the observation that in the absence of added biotin some growth occurred at pH 6.0, in contrast to little or no growth at pH 6.8. Furthermore, the growth could be increased at both pH conditions by the addition of a surfactant. These observations were explained on the assumption that the basal medium contained minute amounts of biotin. The entrance of the biotin into the cells was presumably facilitated by the lower pH and by the surfactant.

Earlier, H. P. Broquist and E. E. Snell (*Arch. Biochem. & Biophys.* **46,** *432 (1953)*) found that *Lactobacillus arabinosus* failed to respond to oleic acid when avidin was added to the medium. In the light of the work of Traub and Lichstein (*loc. cit.*) one might suggest that the avidin prevented the absorption by the cells of the very small amounts of biotin probably present in the medium.

More specific evidence for the role of various factors in facilitating the absorption of biotin by bacterial cells was provided by H. C. Lichstein and R. B. Ferguson (*J. Biol. Chem.* **233,** *243 (1958)*). They grew *Lactobacillus arabinosus* in a medium containing minimal amounts of biotin. The biotin-deficient organisms were harvested by centrifugation and washed with saline to remove any adherent vitamin. These cells were added to a 0.02 M phosphate buffer together with such compounds as biotin, homobiotin, glucose and iodoacetate. After incubation at 37° for two hours, the cells were recovered by centrifugation, washed three times and a sample thereof hydrolyzed with sulfuric acid. After neutralization, the hydrolysate was assayed for biotin.

The results of five experiments showed that when the biotin-deficient cells were incubated for two hours with 0.01 microgram of biotin per 2 ml. of solution, the cells contained from 7.2 to 20.0 $\times$ 10^{-4} micrograms of biotin. When glucose was added to the above solution to give a concentration of 1 molar, the biotin in the cells ranged from 21.0 to 48.6 $\times$ 10^{-4}. The extra biotin absorbed by the cells as the result of the presence of glucose was not apparent when iodoacetate was also added to the tube. Since iodoacetate inhibits glycolysis, this suggests that the absorption of biotin by the cells requires energy. The absorption of biotin in the absence of glucose probably results from the liberation of energy from some energy-rich compound (adenosine triphosphate) already present in the cell.

The addition of biotin antagonists such as homobiotin (this compound contains five CH_2 groups in the side chain instead of four as in biotin) to the medium containing the biotin reduced the concentration of the vitamin in the cells. The reduction was proportional to the concentration of the antagonist. The molar inhibition ratio of homobiotin to biotin was close to 43,000, which was the value found by M. W. Goldberg *et al.* for *Lactobacillus arabinosus* when growth was the variable studied (*Arch. Biochem.* **14,** *480 (1947)*).

The above studies suggest that the absorption of biotin by *Lactobacillus arabinosus* cells requires an active participation of the glycolytic system and that even in the pres-

ence of the necessary energy, the absorption of the vitamin is inhibited by an antagonist such as homobiotin. This work indicates that the transport across the cell membrane of such water soluble substances as biotin is not a simple diffusion mechanism. The relation between the energy requirements and the facilitation of biotin absorption in the presence of a surfactant still remains to be explained. Additional work in this field should include an extension of the work of Lichstein and Ferguson to the system containing the unsaturated fatty acids to determine whether energy is required for the absorption of biotin when oleic acid or a surfactant is in the medium.

SECONDARY CHANGES IN MAGNESIUM DEFICIENCY

Magnesium deficiency in experimental animals (usually rats) is characterized by vasodilation and hyperemia, hyperexcitability, cardiac arrhythmia, spasticity and tetany (*Present Knowledge in Nutrition*, The Nutrition Foundation, New York, 1956, pp. 32–3). A deficiency induced in calves because of too long restriction to a milk diet is marked by inability to utilize dietary magnesium efficiently, by decrease in growth rate (in some) and by convulsions, which, however, appear to be associated with a concomitant hypocalcemia, since both can be relieved by large doses of vitamin D (*Nutrition Reviews* **16,** *173* (*1958*)). Man does not readily develop a deficiency of this mineral since body stores are usually large and bone may serve as a source of magnesium for use in other tissues (*Ibid.* **15,** *232* (*1957*)). However, one case of tetany in a child has been reported to respond to increased dietary magnesium (*Ibid.* **2,** *189* (*1944*)).

In all cases in which analyses have been made, it has been found that the dietary deficiency does not result in a marked decrease in tissue magnesium other than in bones, teeth and serum. However, alterations in concentrations of other minerals, as has been noted for calcium, appear to occur.

In order to obtain a more thorough understanding of these changes, I. MacIntyre and D. Davidson (*Biochem. J.* **70,** *456* (*1958*)) subjected 15 female rats to a magnesium-deficient diet and, at several intervals, analyzed their muscles, plasma, bones, brains, livers and kidneys for sodium, potassium, calcium and magnesium, using the flame photometer. The results were compared to those from a similar group with supplemental dietary magnesium. Although the control animals appeared normal in all respects, those on the deficient diet developed vasodilation and edema of the nose, ears and paws within 12 days. After about 25 days, they had stopped growing and thereafter lost weight and, after 39 days, had started to succumb to the deficiency.

The magnesium content of the muscles decreased progressively to about 78 per cent of the initial value at 64 days. Muscle potassium also declined in a direct relationship to muscle magnesium. Sodium and chloride, on the other hand, increased significantly, but these changes were found to be entirely extracellular. No significant alterations of these cations were found in kidney, liver or brain, with the exception of an increase in kidney calcium. In plasma, however, the reduction in magnesium was accompanied by a slight increase in sodium and a marked increase in calcium (in contrast to the results with calves). Bone magnesium, as had been reported by previous investigators, declined rapidly to 50 per cent of normal during the first three weeks, and then more slowly.

The authors point out that there is considerable evidence, first, that the cell mitochondria (especially heart) depend on an adequate supply of magnesium for their

integrity (a dietary deficiency leading to uncoupling of oxidative phosphorylation according to J. J. Vitale, M. Nakamura and D. M. Hegsted, *J. Biol. Chem.* **228,** *573 (1957)*) and, second, that these structures are probably responsible for the maintenance of gradients of sodium and potassium and for active transport of ions (W. Bartley and R. E. Davies, *Biochem. J.* **57,** *37 (1954)*). The secondary alterations in electrolytes and in fluid balance may, therefore, stem from this cause.

NUTRITIVE VALUE OF PEANUT PROTEIN

Currently, there is a great deal of interest in developing protein sources useful in augmenting the diet of populations where lack of adequate dietary protein results in severe protein malnutrition, or kwashiorkor in children (*Nutrition Reviews* **14,** *263 (1956)*; **15,** *169 (1957)*).

Most of the oil-bearing seed crops are relatively rich in protein, thus furnishing a significant portion of the protein needs of poultry and livestock in our agricultural practices; their use in human diets is under continuing investigation. For the effective use of proteins from such oil-bearing seeds as soybean, cottonseed, and the peanut, it is necessary that the flours prepared from them be relatively low in fat to insure keeping quality under ordinary storage conditions.

The high fat (oil) content of peanuts (45 to 50 per cent) compared to soybean and cottonseed (20 per cent) makes the extraction of oil difficult in a single operation. Since most oil-extraction processes involve the application of heat, and since excessive heat generally affects protein quality adversely, it is of importance to seek improvements in processing procedures to insure a product of uniformly high quality.

Workers at the Indian Institute of Science, Bangalore, India, have investigated the nutritive value of peanut meals prepared by various processing methods. The effects of amino acid, antibiotic, and vitamin B_{12} supplementation were also investigated (S. Balasundaram, H. R. Cama, D. A. Malik and C. Venkateshan, *J. Nutrition* **66,** *75 (1958)*).

Four processing methods were used on a source of Spanish variety peanuts as follows: Meal A was cold solvent-extracted by passing pulverized peanuts through a 40-mesh sieve, removing most of the oil with pressure (4,000–5,000 pounds/sq. in.), and then shaking the residual meal five to six times with low-boiling petroleum ether (40 to 60 degrees C.). The air-dried product (whitish in color) contained 45 per cent of protein, 1.5 per cent of oil, and 9.2 per cent of moisture. Meal B was prepared with the commonly used screw press after slight cooking. It was brown in color and contained 47 per cent of protein, 10.5 per cent of oil, and 7.3 per cent of moisture. Meal C was prepared by screw-pressing and then extracting with trichlorethylene. The extracted meal was steamed to remove residual solvent (open steam released from 25 pounds compression for one-half hour). It contained 52.3 per cent of protein, 1.15 per cent of oil, and 4.3 per cent of moisture. Meal D was the same as Meal C except that steam treatment was more intensive (100 pounds compression for one hour). The product was dark brown and contained 52.3 per cent of protein, 1.48 per cent of oil, and 5.3 per cent of moisture.

Growth tests and nitrogen balance tests were conducted with weanling rats to evaluate the four meals. The test diet for growth evaluation contained 15 per cent of protein (from the test peanut meals), sucrose, salts, peanut oil and starch and was supplemented with vitamins. Protein efficiency ratios indicated that Meals B and C (light heat or steam treatment combined with solvent extraction) were superior to Meals A (raw) and

D (excessive steam treatment). The grams of body weight gain per gram of protein consumed were 1.11, 1.43, 1.44 and 1.38, for Meals A, B, C, and D, respectively.

The biological value data yielded similar results with the meals aligned in the same order: 51.4, 60.2, 63.9, and 53.0, for Meals A, B, C, and D, respectively. Protein values (biological value times true digestibility) were 46.8, 56.2, 57.7, and 48.7, for Meals A, B, C, and D, respectively.

It is evident that the various processing conditions employed affected the nutritive value of the proteins as determined by growth tests and by digestibility studies. The significant enhancement of nutritive value accomplished by gentle heat treatment (Meals B and C) suggests that gentle heat destroyed the trypsin inhibitor which has been reported to be present in unheated peanut meals (R. Borchers and C. W. Ackerson, *Arch. Biochem.* **13,** *291* (*1947*)).

Supplementation of the peanut meals with amino acids indicated that the first limiting amino acid was methionine, a confirmation of earlier studies by C. R. Grau (*J. Nutrition* **32,** *303* (*1946*)). Lysine, isoleucine, or threonine supplementation further improved the protein efficiency ratios, whereas cystine or tryptophan were of no supplementary value.

Supplementation of the raw meal (Meal A) diet with an antibiotic (penicillin, 0.02 per cent) resulted in growth improvement as well as in significant improvement in biological value. Aureomycin and streptomycin, although not growth stimulatory, produced some improvement in the biological value of the raw peanut protein. A similar response was also obtained with vitamin B_{12} supplementation of the raw meal diet (45 micrograms per kilogram).

The response obtained with antibiotic in raw peanut meal diets calls attention to the growth improvement noted by others when raw soybean protein diets are supplemented with antibiotics (R. Borchers, D. Mohammad-Abadi, and J. M. Weaver, *J. Ag. Food Chem.* **5,** *371* (*1957*)).

The peanut meals used by the Indian workers in the described biological tests were subjected to water and buffered aqueous extraction, and the extracts were analyzed electrophoretically by the moving boundary electrophoretic method (H. R. Cama, D. A. Malik and R. Nath, *J. Nutrition* **66,** *123* (*1958*)). The methods of processing significantly affected the relative protein pattern. In the water-extract of meals A and B, for example, there were four components in the ascending limb of the electrophoretic pattern. The raw meal (Meal A), however, contained twice as much of the first component as did the mildly heat-treated Meal B. The excessively heat-treated meal (Meal D) was characterized by the absence of the first component and a marked increase in the last component of the ascending limb of the electrophoretic pattern.

These interesting observations clearly indicate that relatively mild processing procedures have a significant influence on the physical characteristics of peanut proteins and that electrophoretic characterization may prove useful in evaluating processing methods.

The interest in peanut protein as a supplement to low protein diets is emphasized further by the recent dietary studies with African children (E. M. DeMaeyer and H. Vanderborght, *J. Nutrition* **65,** *335* (*1958*)). Based on nitrogen balance studies in children who formerly had kwashiorkor and had gone through successful treatment, a combination of peanuts and beans as a protein supplement to a native diet resulted in nitrogen retentions approaching those obtained with cows' milk as the protein supplement. (The native diet to which the protein supplement was added consisted of rice bread, banana flour, butter, palm oil, sugar, and fruits.)

These important studies will, no doubt, receive much attention among investigators throughout the world. Peanuts can be grown in many parts of the world where at present the human population is suffering from an inadequate protein intake.

GALACTOSE METABOLISM

The conversion of galactose to glucose in biological systems has been studied in detail by Leloir and his associates (*Phosphorus Metabolism, Baltimore* **1,** *67 (1951)*) and by Kalckar and his associates (*Science* **125,** *105 1957*)). Kalckar (*loc. cit.*) has reviewed in some detail the pathway of this conversion which takes place in several steps.

Initially galactose is converted in the presence of the enzyme, galactokinase, and the high energy compound, adenosine triphosphate (ATP), to galactose-1-phosphate. The galactose-1-phosphate then reacts with one mole of the nucleotide, uridine diphosphate glucose (UDPG), under the influence of the enzyme, galactose-1-phosphate uridyltransferase, in the following manner:

Galactose-1-P + UDPG

$\rightleftharpoons$UDP-galactose + glucose-1-P.

The enzyme, uridine diphosphate galactose-4-epimerase, then catalyzes the conversion of the galactose to glucose (UDP-Gal $\rightleftharpoons$ UDPG.).

The conversion of UDPC to uridine triphosphate and glucose-1-phosphate is catalyzed by the enzyme, uridine diphosphate glucose pyrophosphorylase, as follows:

UDPG + PP $\rightleftharpoons$ UTP + glucose-1-P.

The end result of these reactions is that galactose ingested and absorbed into the body is converted into glucose-1-phosphate.

As a result of these studies it has been possible to demonstrate (*Isselbacher et al., Science* **123,** *635 (1956)*) that the disease galactosemia, an inborn error of metabolism in which the body is unable to utilize galactose efficiently, is caused by a deficiency of the enzyme galactose-1-phosphate uridyltransferase. The lack of this enzyme results in the accumulation of galactose-1-phosphate, and the continued ingestion of galactose by those so afflicted can produce mental retardation, cataract formation, cirrhosis and even death.

An extension of these studies by K. J. Isselbacher (*J. Biol. Chem.* **232,** *429 (1958)*) carries our understanding of this disease one step further. It had been known (*Kalckar, et al., Nature* **172,** *1038 (1953)*) that in yeast galactose-1-phosphate could be converted, in the presence of uridine triphosphate (UTP), to uridine diphosphate galactose as follows:

UTP + galactose-1-P $\rightleftharpoons$ UDP-Gal + PP.

This reaction does not require the enzyme galactose-1-phosphate uridyltransferase which is lacking in galactosemic subjects. Also evidence had been obtained that galactesemic subjects can utilize some galactose (*Eisenberg, et al., Science* **125,** *116 (1957)*) and that their tolerance of galactose appears to increase with age (*Townsend, et al., Pediatrics* **7,** *760 (1951)*). In view of these observations Isselbacher (*loc. cit.*) undertook to determine whether mammalian tissues could utilize uridine triphosphate directly for the formation of UDP-Galactose.

The assay procedure consisted of incubating the enzyme preparation together with uridine triphosphate and C^{14}-labeled galactose-1-phosphate in a suitable buffer at 38°C for 30 minutes. At the end of the incubation the protein was precipitated with trichloroacetic acid and the precipitate was removed. The supernatant solution, after adjustment of the pH, was treated with charcoal which adsorbed the nucleotides. These were then eluted with ammoniacal ethanol and the radioactivity was counted. The identity of the radioactive nucleotide as UDP-galactose was confirmed by chromatographic methods. The enzyme preparations were obtained by differential centrifugation of tissue homogenates. Enzyme activity was found mainly in the supernatant solution and in the nuclei of both rat liver and human liver, obtained by biopsy.

When the distribution of the enzyme in rat tissues was studied activity was found in liver, brain, lung and muscle and to a lesser extent in kidney and intestine.

The major problem then to be solved was whether the UDP-galactose might not be arising indirectly. If the preparations were contaminated with glucose-1-phosphate in catalytic amounts, or a source of glucose-1-phosphate such as glycogen, and the enzyme galactose-1-phosphate uridyltransferase, which is found in mammalian tissues, UDP-galactose could arise through an initial reaction of the uridine triphosphate in the incubation mixture with glucose-1-phosphate to give UDP-glucose, as shown in the equation

$$\text{UTP} + \text{glucose-1-P} \rightleftharpoons \text{UDPG} + \text{PP}.$$

The reaction of galactose-1-phosphate with UDPG, as outlined above, would then give UDP-galactose.

In order to examine this possibility the enzyme from beef liver was purified using ammonium sulfate fractionation and dialysis. An active enzyme preparation was obtained in which it was not possible to detect glycogen, glucose-1-phosphate or UDPG. In order to obtain further evidence that the formation of UDP-galactose did not depend on the presence of the system described above, further purification was done. This consisted of salt fractionation, solvent fractionation, and electrophoresis. In this way a highly purified enzyme preparation was obtained and Isselbacher was able to demonstrate that the galactose-1-phosphate uridyltransferase could be separated very largely from the enzyme, uridine diphosphate galactose pyrophosphorylase, which catalyzes the direct conversion of galactose-1-phosphate to UDP-galactose in the presence of uridine triphosphate. He also studied a number of the properties of the enzyme and was able to show that the disappearance of uridine triphosphate and galactose-1-phosphate was matched by the appearance of approximately equimolar amounts of UDP-galactose and pyrophosphate.

This work demonstrates the existence, in mammalian tissues, of two pathways for the conversion of galactose-1-phosphate to uridine diphosphate galactose. One of these is catalyzed by the enzyme galactose-1-phosphate uridyltransferase, an enzyme which is lacking in the disease galactosemia. The other is catalyzed by the enzyme uridine diphosphate galactose pyrophosphorylase, which permits the direct formation of uridine diphosphate galactose from uridine triphosphate and galactose-1-phosphate. Both of these enzymes are present in low concentrations in fetal and neo-natal liver tissue but increase in concentration as the body matures (Isselbacher, *Science* **126,** *625 (1957)*). It appears probable, then, that symptoms in galactosemic infants are severe because of the lack of one of these pathways and a low activity of the other. The improvement in the ability of galactosemic individuals to handle galactose as they mature could then, as the author points out, be a result of an increase in the activity of the enzyme participating in the remaining pathway.

LYSINE, PHOSPHATES AND EXPERIMENTAL CARIES

In most studies on the production of tooth decay in rats, the lesions occur almost exclusively in the sulci on the occlusal surfaces of the molar teeth. Ordinarily the smooth buccal and lingual surfaces are not susceptible to the initiation of carious lesions.

An exception to this generalization was reported by F. J. McClure (*Science* **116,** *229 (1952)*), who observed a high incidence of lesions on the smooth surfaces of the molars when a diet composed largely of processed cereal foods was used. Later he found that the use of roller processed skimmilk in this diet resulted in a higher incidence of this type of lesion than when spray dried skimmilk was used (F. J. McClure and J. E. Folk, *Proc. Soc. Exp. Biol. Med.* **83,** *21 (1953)*). If either of these skimmilk powders were autoclaved

under dry conditions for short periods, the ability to cause smooth surface lesions was increased dramatically.

The addition of 1.5 per cent L-lysine to the diet, or to the drinking water, or the administration of a comparable amount of L-lysine by intubation caused major reductions in the incidence of lesions on the smooth surfaces of the molars (F. J. McClure, *Ibid.* **96,** *631 (1957)*). The intraperitoneal injection of L-lysine resulted in major increases in the rate of growth but did not cause as great reductions in dental caries incidence as administration by the other three routes. The fact that the administration of L-lysine by stomach tube was cariostatic suggests that the route of action for the L-lysine was systemic rather than through a direct oral environmental pathway. However, it is interesting and seemingly contradictory that injected L-lysine was less effective.

Later, F. J. McClure (*J. Nutrition* **65,** *619 (1958)*) described a series of diets, each of which was capable of producing extensive carious lesions on the smooth surfaces of the molar teeth of rats. These diets were composed of wheat flour, dry or toasted bread, or shredded wheat biscuit, cerelose or lactose and liver powder, and were deficient in available lysine to varying degrees. Without mineral supplementation, these diets contained as little as 0.03 per cent calcium and 0.21 per cent phosphorus and some had as little as 9.2 per cent protein. One of the principal diets used was composed of: ground whole wheat 80 per cent, cerelose 18 per cent, and liver powder 2 per cent. When this diet was fed to rats, a high incidence of lesions occurred on the smooth surface of the molars. Complete replacement of the whole wheat by ground shredded wheat biscuit, by a commercial whole wheat flour, by white bread or by toasted white bread, did not materially alter the severity of caries from the level observed when ground whole wheat was used. When the ground whole wheat alone or the ground whole wheat plus cerelose, or commercial whole wheat flour plus lactose were autoclaved prior to ration mixing, major increases in the caries incidence occurred. When the diets containing toasted white bread or the autoclaved mixture of commercial whole wheat flour and lactose were supplemented with 2.0 per cent L-lysine, major reductions in the severity of carious lesions were observed.

The addition of 2.9 per cent ash of powdered skimmilk, of 1.1 per cent calcium carbonate and 0.6 per cent dibasic sodium phosphate or of 1.6 per cent dibasic sodium phosphate alone to one of the diets containing commercial whole wheat flour, or ground whole wheat or ground shredded wheat biscuits caused significant reductions in carious lesions on the smooth surfaces of 56 per cent to 80 per cent. However, the addition of either 1.0 per cent calcium carbonate or 2 per cent dibasic calcium phosphate did not cause any reductions in the incidence of this type of carious lesion. Analyses of the ash, calcium and phosphorus contents of the dentin and enamel of incisor and molar teeth indicated that these compounds were unaffected by the dietary deficiency of calcium or of phosphorus produced by the unsupplemented rations.

In additional studies, F. J. McClure and A. Muller, Jr. (*J. Am. Dent. Assoc.* **58,** *36 (1959)*) added either dibasic sodium phosphate or dibasic calcium phosphate directly to cariogenic diets or to whole wheat flour to be used for baking bread that was to be included in cariogenic diets. It is noteworthy that all the diets in these studies were deficient in lysine and hence were inadequate for normal growth. Unless supplemented, the diets were all extremely low in calcium, and borderline in protein. The diets containing whole wheat flour were borderline in phosphorus while the diets containing bread were even lower in phosphorus. As in the earlier experiments, the carious lesions studied were primarily of the smooth surface type with few occlusal lesions.

In the first experiment, offspring of two strains of rats were used, the one from the Naval Medical Research Center and the other from the National Institutes of Health. The basal diet consisted in per cent of: whole wheat flour 80, cerelose 18 and liver powder 2. When 2 per cent of dibasic sodium phosphate was added to the diet, the average severity of caries experienced by the rats of both strains was reduced by highly significant amounts. When 2 per cent of dibasic sodium phosphate was supplied to the rats in the drinking water instead of in the diet, the rats of the strain from the National Institutes of Health had almost as great a reduction as when this phosphate was in the diet. However, the supplement in the drinking water of the rats from the strain maintained by the Naval Medical Research Center had no effect on the incidence and severity of carious lesions. This difference in results between the two strains is interesting and needs further exploration. The higher susceptibility of the strain from the National Institutes of Health may be related to the different response. For the remaining experiments in this series, only the strain of the National Institutes of Health was used.

In the second experiment, the diet fed the control rats had 1.25 per cent of calcium carbonate added at the expense of a comparable weight of whole wheat flour. The rats in the second group received supplements of 1.25 per cent calcium carbonate and 1.5 per cent dibasic sodium phosphate at the expense of whole wheat flour. The rats in the third group received supplements of 0.12 per cent calcium carbonate and 1.5 per cent dibasic calcium phosphate, both at the expense of whole wheat flour. Dental caries on smooth surfaces were almost completely prevented in the rats of the second group by the dietary supplement of dibasic sodium phosphate. The rats in the third group did not benefit by the supplement of dibasic calcium phosphate.

In the third experiment, there were five groups of rats, the first of which received a diet containing a dried ground white bread in place of all the whole wheat flour in the basal diets of the first two experiments. The rats in the second group received the white bread diet supplemented with 1.4 per cent dibasic calcium phosphate while the rats in the third group received the bread diet supplemented with 1.4 per cent of dibasic sodium phosphate. The rats in the fourth group received a diet that contained the same level of an identical bread except that it had been made from a wheat flour to which had been added 2 per cent dibasic calcium phosphate prior to baking. The rats in the fifth group received a diet made from a bread baked from flour containing 2 per cent dibasic sodium phosphate.

The rats in the second group, where the diet was supplemented with dibasic calcium phosphate, had a slight but not statistically significant reduction in dental caries while the rats in the third group where the diet was supplemented with dibasic sodium phosphate had a greater reduction in dental caries. In groups four and five, where the bread flour was supplemented with either dibasic calcium phosphate or dibasic sodium phosphate, moderate reductions in dental caries occurred that were on the borderline of statistical significance. Under the conditions of the supplementation of the bread flour, the sodium and calcium salts appeared to be equally effective.

The authors postulated that the presence of sodium chloride in the bread dough may have resulted in some or all of the relatively insoluble dibasic calcium phosphate being changed into a more soluble phosphate during the baking process. None of these experiments defines the route of action of the phosphate supplement. Since the crowns of the teeth were almost fully formed and calcified at the beginning of the experiments, the mode of action, whether through the oral environment or a systemic pathway, would not involve calcification of the crowns other than through an absorptive or exchange mechanism.

These authors pointed out the potential importance of phosphate supplementation of foodstuffs in the dietary control of human caries. They mentioned an unpublished Swedish study conducted by A. Strålfors who studied the influence of the inclusion of a dibasic calcium phosphate supplement in the bread and sugar of school children's lunches and reportedly observed a strong dental caries inhibition. They also pointed out that the self-rising flours used extensively in the Southern states contain added phosphates. The well known observation that the dental caries incidence in the Southern states is generally lower than that in the Northern states has usually been attributed to greater exposure to sunshine through more intense sunlight, more hours of sunlight per year and a greater area of skin exposure and hence a greater availability of vitamin D through irradiation in the South (C. A. Mills, *J. Dent. Res.* **16,** *417 (1937)*; B. R. East, *Am. J. Public Health* **29,** *777 (1939)*).

In view of the current experimental studies, possibly phosphate consumption may also enter the picture. One of the unresolved problems in McClure's studies is the concurrent lysine deficiency throughout, which is apparently a necessary prerequisite for production of the smooth surface type of carious lesions in rats. If lysine deficiency is essential for the production of these lesions and for a phosphate effect, the possibility of a beneficial influence of phosphate supplements to bread or sugar for use by the human population may be reduced. However, the results with rats are sufficiently encouraging to merit extension into human surveys. In the design of such surveys, a real opportunity exists for the collection of thoroughly correlated nutritional and dental information simultaneously.

VITAMIN D AND CITRATE METABOLISM

Skeletal changes characteristic of vitamin D deficiency are not seen in rats fed a diet lacking in vitamin D if the diet contains adequate quantities of phosphorus or calcium; nevertheless, histologic evidence of rickets can readily be demonstrated if a diet low in phosphorus and lacking vitamin D is fed. Biochemical changes attributable to vitamin D deficiency, such as hypocalcemia and a low concentration of citrate in the plasma, do occur in the rat, however, even when rickets does not develop (Harrison and Harrison, *Yale J. Biol. Med.* **24,** *273 (1952)*; Steenbock and Bellin, *J. Biol. Chem.* **205,** *985 (1953)*).

Experiments on citrate metabolism in rats fed a vitamin D-free diet with an adequate phosphorus content and a normal calcium to phosphorus ratio are described in a report by Helen C. Harrison, H. C. Harrison and E. A. Park (*Am. J. Physiol.* **192,** *432 (1958)*). The objective of the investigation was to obtain evidence about the relationship between calcium and citrate metabolism.

The rats used in the experiments were three weeks old. They were fed a diet containing casein, 18; carbohydrate, 70; peanut oil, 8.0; brewer's yeast, 5.0; salt mixture, 5.0; and vitamin A, 10,000 I.U. The diet contained 0.7 per cent of calcium and 0.56 per cent of phosphorus. After three weeks on the basal diet half of the rats were given a single dose of 100 I.U. of vitamin D_2 orally and groups were killed after 24, 72 and 192 hours. An equal number of control rats were also killed at each interval. Determinations of calcium, phosphorus and citrate were made on serum and on one of the tibia, from each rat. The other tibia was removed for histological examination.

The striking finding in the first experiment was that the serum calcium concentration of rats maintained for only three weeks on this vitamin D-free diet, which contained

an adequate amount of calcium, had fallen from 10 mg. per cent to 6.0 mg. per cent. Within 24 hours following the administration of a single dose of 100 I.U. of vitamin D_2 this had risen to 9 mg. per cent, and two days later, had reached almost 11 mg. per cent, a concentration that was maintained for five more days when the experiment was terminated.

The serum phosphorus level of the deficient rats was 10.0 mg. per cent. This increased following vitamin D administration to 13 mg. per cent, but within eight days had returned to the original, somewhat lower, value. The citrate concentration in the serum was low (3.0 mg. per cent) in the deficient rats. It did not rise as rapidly as did the serum calcium concentration following vitamin D therapy, but had risen to 6.4 mg. per cent within three days. Within eight days after vitamin D was administered this had fallen almost to the original value. The bone citrate values paralleled quite closely those for serum up to three days but remained high on the eighth day, despite the fall in the serum level. The values for bone calcium were constant throughout the experimental period.

In a second experiment the effects of treatment of vitamin D-deficient rats with parathyroid extract, or with cortisone, were determined. Parathyroid extract (two doses of 100 units, each administered subcutaneously 18 hours apart) failed to influence either the calcium or the phosphorus concentration of the serum or the calcium content of the tibia unless vitamin D was administered to the rats prior to the parathyroid treatment. In the vitamin D-treated rats parathyroid injections caused a fall of 5.0 mg. per cent in serum phosphorus concentration and a rise of 2.5 mg. per cent in the calcium concentration, as compared to values for control rats given only vitamin D. The parathyroid extract did not influence the serum citrate values.

Another group of vitamin D-deficient rats was given 30 mg. of cortisol per 100 g. of diet for nine days, then 100 I.U. of vitamin D_2 three days before they were examined. Cortisol treatment depressed the citrate concentration in both the serum and the bone of the deficient rats and suppressed the accumulation of citrate caused by vitamin D treatment. The serum citrate concentration of cortisol-treated rats was just above 1 mg. per cent whether or not they received vitamin D. Cortisol did not alter the effect of vitamin D in raising the serum calcium concentration.

The histological studies revealed only slight differences between the bones of the vitamin D-treated, and the vitamin D-deficient, rats. There was, however, a definite retardation of growth when vitamin D was not provided. The body weight of the rats given vitamin D was 138 g. after three weeks, and this was reduced to 109 g. when vitamin D was omitted from the diet. The lower rate of growth was accompanied by lighter tibial weight.

These results indicate, as the authors state, that there is a definite difference between the response of the rat and that of the human infant to vitamin D deficiency. The rat maintains a high serum phosphorus concentration when the diet contains adequate calcium and phosphorus, whereas the infant fed a similar diet usually develops hypophosphatemia. The authors suggest that the high serum phosphate concentration may permit normal calcium deposition in the bone until the serum calcium concentration is severely reduced. This, together with lowered calcium absorption, could account for the severe hypocalcemia in the rat. It has also been suggested that vitamin D is required for mobilization of bone calcium to maintain serum calcium levels (Carlsson, *Acta physiol. Scandinav.* **26,** *212 (1952)*) and this, too, may be an important factor. It would also appear from these results that vitamin D is required for the expression of parathyroid hormone action.

The effects observed with cortisol are of particular interest because they indicate that the action of vitamin D in restoring

serum calcium levels and in stimulating bone calcification can be separated from its effect on citrate metabolism. It is evident that cortisone treatment prevented the increase in citrate concentration usually seen when vitamin D-deficient rats are given vitamin D, whereas the elevation of serum calcium concentration was unaffected.

It is tempting to pose questions. Does vitamin D have two distinct metabolic functions, one of which is inhibited by cortisol? From the structural similarity of cortisol and vitamin D, is the suppression of the effect of vitamin D on citrate metabolism by cortisol conceivably a case of structural antagonism?

GLYCINE AND AMINOETHANOL IN THE CHICK

Although the weanling rat has a nutritional requirement of ten essential amino acids, the chick requires dietary glycine in addition to these. The earlier demonstrated need for dietary glycine by the young chick (H. J. Almquist and E. Mecchi, *J. Biol. Chem.* **135,** *355* (*1940*)) and its interrelationships with dietary arginine for creatine formation have been confirmed by recent studies (A. W. Wietlake *et al.*, *J. Nutrition* **52,** *311* (*1954*); A. G. Hogan *et al.*, *J. Nutrition* **62,** *97* (*1957*)). However, R. L. Wixan, G. E. Pipkin, J. H. Wikman and P. L. Day of the University of Arkansas (*J. Nutrition* **63,** *13* (*1958*)) have inquired more closely into the specificity of the glycine requirement.

Previous experiments had established that serine could substitute for glycine as measured by the criterion of growth. In addition to interconversion of glycine and serine, the authors were of the opinion that there must be some pathway for their formation as evident from the normal growth of rats on diets devoid of serine and glycine.

Investigations of others have established the following biosynthetic pathways in the rat: betaine to dimethyl-glycine to sarcosine to glycine; aminoethanol to glycolaldehyde to glycolic acid to glyoxylic acid to glycine; and triose to hydroxypyruvate to serine. These pathways have been examined in the chick by Wixan *et al.* (*loc. cit.*) and the results discussed with respect to these metabolic reactions in the two species.

Before planning experiments with compounds possibly related to glycine it was necessary to study the specificity of the glycine nitrogen requirement. The addition of 1.78 per cent DL-alanine or 2.94 per cent L-glutamic acid did not improve the chick growth rate in the same manner as did the 1.50 per cent glycine supplement. The addition of 2.28 per cent diammonium citrate also failed to give growth comparable to that with the glycine supplement.

The authors noted that in the above two experiments the feed efficiency was highest for the glycine-supplemented chicks. With the other test substances, the feed efficiency was related directly to the rate of growth. They therefore conclude that the attainment of maximum growth is not restricted by a slight deficiency of total nitrogen in the diet. The utilization of the extra dietary nitrogen in the test substances is probably related to the carbon skeleton attached to the nitrogen. Apparently then, the rate of synthesis of the carbon chain of either glycine or serine limits the rate of growth in chicks.

Since 2.10 per cent of DL-serine improved the growth rate of glycine-deficient chicks to the same degree as 1.50 per glycine, there seems to be either a reversible metabolic transformation of serine and glycine or a multiple essential amino acid deficiency, namely glycine and serine. If there were such a multiple requirement, the addition of serine plus glycine should implement an increase in growth which is greater than when either is given alone. There was, however no improvement in rate of growth when the supplement consisted of simultaneous addi-

tion of 1.50 per cent glycine and 2.10 per cent DL-serine.

Earlier experiments by other investigators with the rat and rabbit suggested the need to test the possible metabolic conversion of tri-, di- and monomethylated glycine to glycine in the chick. It was found that the growth rates, and, hence, feed efficiencies, of diets containing betaine, dimethyl-glycine and sarcosine were no better than when the basal diet was employed. These results are in sharp contrast to the known presence of these metabolic reactions in the rat.

Isotope and enzyme studies have demonstrated that in the rat, choline must first undergo oxidation to betaine, which, after methylation, yields dimethylglycine (J. W. Dubnoff, *Arch. Biochem.* **24,** *251* (*1949*)). Both oxidation and transmethylation to form methionine proceed as readily in liver homogenates from the chick as in those from the rat (Dubnoff, *loc. cit.*). Since the transfer of a methyl group away from betaine can occur readily, a later step must be rate-limiting for possible glycine formation in the chick.

In summary of the voluminous evidence on the subject, there is remarkable agreement regarding species differences with respect to the rate of metabolism of the methylated glycines. The rat can carry out all steps of the conversion of betaine to glycine; the rabbit can complete only the last step of oxidation of sarcosine to glycine, and, although the chicken can readily transfer one methyl group of betaine, it cannot perform the last two reactions at a measurable rate.

Evidence for the reversible reactions between glycolic acid, glyoxylic acid and glycine in the rat has been described (L. D. Abbott Jr. and H. B. Lewis, *J. Biol. Chem.* **137,** *535* (*1941*)). To determine if these reactions also occur in the chicken, glycine, sodium glyoxylate and glycolic acid were added to basal diets deficient in glycine. In contrast to glycine, glyoxylic acid and glycolic acid gave a depression of the rate of growth.

Studies with radioactive aminoethanol have indicated that this substance is utilized for glycine synthesis in the rat (D. Stetten, Jr. *J. Biol. Chem.* **140,** *143* (*1941*); L. O. Pilgeram, *et al.*, *J. Biol. Chem.* **204,** *367* (*1953*)). Surprisingly, the addition of aminoethanol to a glycine-deficient basal ration led to an increase in the chick of the same magnitude as that resulting from glycine supplementation.

The carbon skeleton of aminoethanol may form the two-carbon chain of glycine by two pathways, via choline or via glycolaldehyde. Both of these obtain in the rat. Since the addition of either N-monomethylamine-ethanol or N,N^1 - dimethylamino - ethanol failed to improve the growth rate of chicks it was concluded by the authors that the pathway of deamination of aminoethanol, oxidation of the aldehyde carbon of glycolaldehyde, oxidation of the carbinol carbon of glycolic acid, and their transamination to produce glycine must be the quantitatively important reaction in the chick.

AMINO ACID IMBALANCE AND TOTAL FOOD CONSUMPTION

When rats subsisting on a diet barely adequate in protein or containing an incomplete protein are given supplements of specific amino acids, the result may be a decrease in growth rate and nitrogen retention. This is particularly common in cases in which the second most limiting amino acid is increased rather than the most limiting one. Such an effect, if it can be prevented by additional supplementation with the most limiting amino acid (or acids), is termed "amino acid imbalance" (A. E. Harper, *Nutrition Reviews* **14,** *225* (*1956*); *Ann. N. Y. Acad. Sci.* **69,** *1025* (*1958*)).

The exact mechanism by which this effect is produced, however, has not been apparent. Several laboratories are concerned with this problem, which needs elucidation, especially in view of the present availability of amino acids for use in supplementing the incomplete proteins consumed in many countries.

P. D. Deshpande, A. E. Harper and C. A. Elvehjem (*J. Biol. Chem.* **230,** *327, 335 (1958)*) have carried out experiments in which weanling rats were fed diets containing only 6 per cent of the protein, fibrin, in which several amino acids appear to be limiting. Thus, supplementation with methionine and phenylalanine precipitated symptoms (decreased growth and nitrogen retention) which could be prevented by supplemental leucine, isoleucine, valine and histidine. A further symptom of amino acid imbalance was a drop in food intake, which probably served to minimize the detrimental effect of the imbalanced diet. However, forced-feeding of the deficient diet resulted in early death of the rats.

In an effort to increase food consumption of the rats on the imbalanced diet and thus to magnify the effect of the imbalance without the trauma of forced feeding, it was thought that "spaced feeding" (*Nutrition Reviews* **15,** *26 (1957)*), in which animals are trained to consume all their food during short periods, might be useful. Consequently an experiment was designed in which the conditions employed previously with the weanling rats were repeated with adult animals, with the condition of spaced feeding added (U. S. Kumta, A. E. Harper and C. A. Elvehjem, *J. Biol. Chem.* **233,** *1505 (1958)*).

For this experiment, four diets were employed; a basal low-protein diet (A), containing 6 per cent of fibrin as the protein; an imbalanced diet (B), identical to diet A with additional 0.4 per cent DL-methionine and 0.6 per cent DL-phenylalanine; a corrected diet (C), identical to B but with additional 0.2 per cent L-leucine, 0.2 per cent DL-isoleucine, 0.4 per cent DL-valine and 0.6 per cent L-histidine hydrochloride; and a protein-free diet identical to A but with the fibrin replaced by dextrin.

Groups of six adult male rats were fed diet A for four to five days and then placed on the various experimental diets. Two groups were trained to consume their daily ration of diets A and B, respectively, in two hours (spaced-feeding groups). Three groups (protein-depleted groups) were kept on the protein-free diet for one week and were then fed the diets A, B or C. Rats trained to spaced-feeding consumed less of diet B and grew at a lower rate than on diet A. The protein-depleted group also grew more slowly on diet B. Weight gain of the depleted group was normal on diet C, while that of a non-depleted group was even augmented.

In the study of nitrogen balance, pair-fed control rats were used to compensate for the decreased food intake of animals on diet B. In all cases, nitrogen intake and retention were considerably lower on diet B than on diet A. The pair-fed control rats were similar to experimental animals except for a tendency to even lower nitrogen retention than the experimental animals consuming diet B *ad libitum*. In the case of the spaced-fed rats, however, the experimental animals consumed an amount of diet B on the first day approximately the same as the amount of A consumed on the previous day. Nitrogen retention, as would be expected, remained high until the second day when food consumption dropped.

From the results of these experiments, the authors were able to draw several conclusions.

First, since the spaced-fed animals, which were trained to consume large amounts of food in a short time, ate normal amounts of diet B on the first day and only reduced their food intake on the second day, the effect of the imbalanced diet was in some metabolic disorder rather than in impalatability.

Second, since pair-fed control animals

had even lower nitrogen retention than the experimental rats, the immediate cause of this symptom may have been the decreased food intake, which is known to have such an effect.

Third, since diets A and C produced about the same amount of growth, it seems possible that the two additional amino acids of diet B (methionine and phenylalanine) decreased utilization of the four amino acids (isoleucine, leucine, histidine and valine) which, in diet C, corrected the imbalance.

Although these studies have not yet succeeded in definitely pinpointing the basic disorder in amino acid imbalance, they are not inconsistent with the idea advanced by several workers (A. E. Harper, *loc. cit.*) that the feeding of an imbalanced diet increases the breakdown of the tissue proteins and thus results in increased nitrogen excretion. However, this may be merely another way of stating the problem and of emphasizing the fact that although a good beginning has been made, the end is not in sight.

DEVELOPMENTAL INFLUENCES ON CARIES SUSCEPTIBILITY

A decade ago serious studies began on the potential relationship between nutritional influences during tooth development in rats and the caries susceptibility of these teeth when fully formed. The primary observation in this area was made in experiments where the consumption of a diet composed of natural foodstuffs by female rats during pregnancy and lactation resulted in the formation of caries-resistant teeth in their offspring, whereas the consumption of a highly purified diet under comparable circumstances led to the formation of more highly caries-susceptible teeth (*Nutrition Reviews* **6,** *196 (1948)*; **8,** *37 (1950)*).

In later experiments it was demonstrable that the fluoride content of the natural diet was insufficient to account for the increased resistance, and that a large part of the increased resistance was attributable to other minerals in the natural diet (*Ibid.* **13,** *29 (1955)*).

In a modification of this original experimental design, R. R. Steinman and M. I. Haley (*J. Dent. Res.* **36,** *532 (1957)*) administered carbohydrate solutions or suspensions to suckling rats as supplements to their mothers' milk. In the first trial two litters were used. Beginning two and three days after birth and continuing until weaning, the six rats in one group received, orally, a 20 per cent sucrose solution three times a day while the other nine received an equal volume of water at the same times. Up to the fifth day, the carbohydrate provided equalled $\frac{1}{1000}$ of the body weight per day and thereafter was increased to $\frac{1}{750}$. For an appropriate period after weaning, all rats in both groups were maintained on a cariogenic diet. At sacrifice the rats fed the sucrose supplement had about two and one-half times the amount of tooth decay observed in the control animals.

A second experiment was conducted in which a comparison of 20 per cent lactose and 20 per cent raw starch was made without control rats. The rats fed the lactose supplement had much less tooth decay than the control animals in the first experiment, but the rats fed the raw starch supplement had almost four times as much tooth decay as the control animals in the first experiment.

In a third experiment, four supplements were compared, again without benefit of control animals: 20 per cent sucrose, 20 per cent lactose, 20 per cent glucose and a mixture of 10 per cent glucose and 10 per cent fructose. The average caries incidence for the rats in the last three groups was closely similar but that of the rats fed the sucrose supplement was much higher, comparable to the value for the rats in the raw starch group of the second experiment, and much

higher than for the sucrose group of the first experiment.

Obviously it is not fair to compare the experimental rats in the second and third experiments with the control animals in the first experiment because of the different litters represented; by reason of the inadequate experimental design no adequate alternative is provided.

In a second series of experiments with similar designs, the influence of intraperitoneal injections of carbohydrates was compared with their oral administration (R. R. Steinman, M. I. Haley and P. O'Day (*J. Dent. Res.* **37,** *719 (1958)*). In the first trial, one group of nine suckling rats was fed a 20 per cent glucose solution at a level to provide 1 mg. of glucose three times a day from the third day of age until weaning. A group of littermates was provided the same amount of glucose by three intraperitoneal injections per day. After a suitable post-weaning period on a cariogenic diet, the injected rats had significantly more tooth decay than those fed the same amount orally. Unfortunately, again there was no control group to which these values could be related.

In a second trial, there were three groups of rats, one receiving a mixture of 10 per cent glucose and 10 per cent fructose by injection, a second group receiving the same solution by mouth, and the third which served as a control. Both carbohydrate supplemented groups had significantly greater incidences of dental caries than the control group. It is worthy of mention that the rats in this control group had an almost indistinguishable incidence of dental caries from the rats in the carbohydrate-injected group in trial one, and a significantly higher incidence than those receiving the oral carbohydrate supplement.

In the third trial, the rats in three separate groups were given the same level of injection of the same solution as in trial two. While the rats in the first group received the carbohydrate injection only, the rats in groups two and three were provided with either 0.1 mg. of vitamin C per 10 g. of body weight or 0.1 mg. of vitamin B_6 by injection per day. At the end of the assay period, the rats in groups two and three were reported to have significantly lower incidences of dental caries than those in group one. Again no control animals without carbohydrate supplementation were included in the experimental design.

In another series of studies, efforts were made to test whether various systemic influences, imposed after tooth development was largely completed, would alter the initiation and progression of carious lesions (R. R. Steinman and M. L. Hardinge, *J. Dent. Res.* **37,** *784 (1958)*). A comparison was sought of the "circulation" within the dentin by means of trypan blue injections in rats fed either laboratory chow or a cariogenic diet after weaning. It was reported that there was a greater "circulation" within the dentin of the chow-fed rats and that the secondary dentin of the rats fed the cariogenic diet had a series of irregular wavy concentric rings.

In a second experiment, the post-weaning supplementation of a ration said to represent a distribution of natural foodstuffs comparable to a typical American diet with the low level of 16 mg. of pyridoxine per kilogram resulted in a statistically significant reduction in dental caries.

In a third experiment, the same typical American diet with the omission of the dessert items was used as the basal ration. Supplementation of one group of littermates by intraperitoneal injections of a solution of 10 per cent glucose and 10 per cent fructose at a level of 1 mg. of carbohydrate per gram of body weight three times a day resulted in a 2.9-fold increase in dental caries, a highly significant increase.

Although, obviously, these data are not strictly comparable from one experiment to another by reason of the different litters, and hence the different hereditary characteristics represented, the control rats in the second experiment had an average caries score that was 2.3 times greater than that of the control animals in the third experi-

ment where the dessert items were omitted. Thus, the intraperitoneal injection of these amounts of carbohydrate appeared to be as cariogenic as the oral consumption of the desserts.

In another experiment, eight experimental groups of rats were fed various combinations of human foods over a three-generation period while a control group of rats was fed a high-sucrose purified diet for the same period (R. R. Steinman, M. G. Hardinge and R. W. Woods, *J. Dent. Res.* **37,** *865 (1958)*). Six food groups were described: a basic group A composed of 14 varied foodstuffs, a refined cereal group B composed of white enriched bread and refined corn meal, a whole grain cereal group C containing whole wheat bread and unbolted corn meal, a dessert group D containing plain white iced cake and lemon merinque pie, a fruit group E composed of apple, orange and banana, and a fruit and a vegetable category, F, containing carrots, cabbage, apple, orange and banana.

All rats in the eight experimental groups received basic food group A and one or two other food groups. The desire in these food combinations was to obtain mixtures of human food that would somewhat approximate human dietaries, albeit of necessity from a much more restricted choice of foodstuffs than would be typical in the human population. The food components were ground and mixed thoroughly. Each group of rats in the first generation consisted of 13 subjects, for each of the second and third generations three females and one male were selected to be the parents in each of the subject groupings. In each of the second and third generations, breeding was stopped when 13 offspring were available for the new generation in each group.

The nutritional status of the rats from generation to generation was appraised by noting weaning weights, weekly weight gain, total body weight, length of mandible, ability to reproduce and general physical appearance. A value from 1 to 100 was assigned for each of these parameters; the average nutritional state of each group of rats was determined and called the "nutritional index". Caries scores were similarly rated in order to give the "caries index". The exact means by which these indices were determined was not described; their validity and their value were also left in doubt.

The rats fed a mixture of food groups A, C and F had the poorest nutritional index throughout all three generations and the highest caries scores in the second and third generations. The rats fed a mixture of food groups A and B, the rats fed a mixture of food groups A and C, and the rats fed the synthetic diet, had the best nutritional statures and the lowest caries scores.

The other groups were intermediate both with respect to nutritional index and caries score. The authors concluded that there was an association between good nutritional status and low caries incidence. While their average data point strongly toward this conclusion, the question arises whether the data are based upon sufficient numbers of animals in each generation to merit the confidence placed upon the statistical evaluation. This query appears to be particularly pertinent ln view of the lack of any obvious correlation between the content of the major nutrients in the several diets and the actual nutritional status of the offspring.

This series of investigations by Steinman and his collaborators raises a number of thought-provoking questions for which answers are presently unavailable. The supplementation of suckling rats with carbohydrates with or without an added vitamin is a novel procedure with which to attempt to study the influence of nutrition on tooth development. Admittedly the problem of exact control in this type of problem is very difficult; yet, without suitable controls interpretation of the results is virtually impossible. It is to be hoped that these studies will be extended and intensified until satisfactory answers are found for the stimulating questions raised by the present series.

NOTES

Letter to the Editor

Gentlemen:

I was pleased to see attention directed to the broad nutritional significance of investigations using algae in your article "Nutrition of Algae" (*Nutrition Reviews* **17,** *16 (1959)*).

The introductory clause of the last paragraph of that article, however, implies an anabolic inertness of ammonia and urea in animal tissues which is sharply contradicted by the data of Rose and Decker (*J. Biol. Chem.* **223,** *107 (1956)*). Here extensive synthesis of non-essential amino acids using dietary urea-nitrogen was demonstrated in rats using diets containing only limited amounts of the essential amino acids. This observation is of immediate theoretical importance. N. S. Scrimshaw, G. Arroyave, and R. Bressani (*Ann. Rev. Biochem.* **27,** *403 (1958)*) in discussing the nutritive value of urea cite similar studies using hamsters (T. Matsumoto, *J. Agr. Research* **6,** *127 (1955)*) and humans (W. C. Rose and R. L. Wixom, *J. Biol. Chem.* **217,** *997 (1955)*; L. E. Holt and S. E. Snyderman, *Some Aspects of Amino Acid Supplementation*, Rutgers University Press, New Brunswick, N. J. (1956)).

Commercial exploitation of urea and ammonia as protein sources in ruminant rations is already a fact. It is conceivable that when the metabolic bases of amino acid imbalance phenomena have been elucidated, non-protein nitrogenous nutrients may prove useful even in the diets of monogastric animals.

Sincerely yours,
KENDALL W. KING
Associate Professor of Bacteriology
Virginia Polytechnic Institute

Pyridoxine- and Niacin-Deficiency Anemias in Man

Uncomplicated deficiency of a single member of the B-complex in man is quite rare. Partially, this is due to the fact that if the natural diet is deficient in one of the B-vitamins it is likely to be deficient in several; partially, it is due to the reluctance of experimentors to carry vitamin deficiency in human subjects to a critical point either with deficient diets or with vitamin antagonists. In particular, pyridoxine deficiency in man is rarely seen naturally, and only with the use of the antagonist, desoxypyridoxine, has it been possible to produce much more than subjective symptoms experimentally (*Nutrition Reviews* **7,** *93 (1949)*; **12,** *10 (1954)*).

An accompanying symptom of deficiency of several of the B vitamins in experimental animals is anemia. A macrocytic anemia, for example, is observed in niacin-deficient dogs and a microcytic anemia is seen in pyridoxine-deficient dogs and pigs (*Nutrition Reviews* **3,** *29 (1945)*). This is not unexpected in view of the multiplicity of functions of these vitamins and the complexity of hemoglobin synthesis. However, anemia was not reported as a symptom of experimental pyridoxine deficiency in human subjects, and the anemia reported in human pellagra has been ascribed to a deficiency of folacin (W. A. Krehl, N. Torbet, J. de la Nuerga and C. Elvehjem, *Arch. Biochem.* **11,** *363 (1946)*).

Two cases of anemia associated with vitamin deficiency have recently been reported by H. Fox and A. Kondi of the Wellcome Trust Research Laboratories, Nairobi, Kenya (*Blood* **13,** *1054 (1958)*). The first, an apparently well-nourished man of 35, complained of weakness, pain in the legs, edema, breathlessness, palpitation and

headache. Laboratory findings revealed, among other things, a red blood cell (R.B.C.) count of 4,133,000/cu. mm., hemoglobin count of 8.3 g./100 ml., hematocrit of 34 per cent, reticulocytes—3.5 per cent and some giant stab cells in the marrow. A program of 30 g. daily of ferrous sulfate for 16 days was accompanied only by still further decreases in hematological values; thus, a course of 20 mg. daily of oral pyridoxine was started. Within three days all values began to rise, and within two weeks the patient had improved to such an extent that he was discharged. During the entire course of treatment, the serum proteins did not change significantly from normal.

The second patient, a woman of 40 years, had typical symptoms of pellagra (skin disorders, mental confusion, and others). Hematological studies revealed an R.B.C. count of 5,300,000/cu. mm., hemoglobin count of 8.6 g./100 ml. and again some giant stab cells in the marrow. As in the other case, oral ferrous sulfate resulted only in a deterioration of her condition. Intravenous injections of 100 mg. daily nicotinic acid brought about remission of the symptoms within four days, and after two weeks on an oral nicotinic acid regimen she was discharged as normal. Serum proteins were again normal throughout the period of observation.

Although the authors indicate that the presence of the giant stab-cells in the marrow of both cases is typically a result of folacin deficiency, this and all other symptoms disappeared during treatment, in the one case with pyridoxine, and in the other with nicotinic acid. It therefore appears that the two cases may have been examples of uncomplicated single vitamin deficiencies.

Normal Catalase Activity in Iron Deficiency

In work previously reviewed (*Nutrition Reviews* **16,** *155 (1958)*), E. Beutler established that cytochrome C levels are reduced in iron-deficient rats without anemia. He had doubted the thesis that the body guards its iron enzymes "tenaciously" in iron deficiency, because of the improvement in symptoms of many patients given iron, before the hemoglobin increased. He thought that such symptoms, as well as the changes in nails, mouth and esophagus, evidently not due to anemia, might be caused by deficiencies of iron-containing enzymes.

E. Beutler and R. K. Blaisdell have studied a second enzyme, catalase, in human erythrocytes, (*J. Clin. Invest.* **37,** *833 (1958)*) and find no decrease in its activity in iron deficiency.

The authors found previous studies on animals and man inconclusive or technically invalid. They compared 11 patients with iron deficiency anemia with nine normal subjects. Erythrocyte catalase activity was measured by the method of von Euler and expressed per ml. of red cells, per 10^{10} red cells and per gram of hemoglobin.

Even with severe iron need, there was no change in the catalase activity per ml. of erythrocytes. The authors conclude that catalase synthesis is more successful than hemoglobin synthesis in competing for a limited quantity of iron. This is the reverse of what Beutler had shown in the case of cytochrome C in iron-deficient rats.

Recent Books

The Healthy Way to Weigh Less. Council on Foods and Nutrition, American Medical Association, 535 North Dearborn Street, Chicago 10, Illinois. Pamphlet, without charge.

The Transactions on the First Conference on The Central Nervous System and Behavior (February 23, 24, 25 and 26, 1958). Editor: Mary A. B. Brazier, Massachusetts General Hospital, Boston. 426 pages, 168 illustrations, 1 table, bibliography, index. Josiah Macy, Jr., Foundation, 16 West 46th Street, New York 36, New York. Price: $5.25.

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